# SDS-PAGE

## Protocol:

### Solutions：

40 % Acrylamide /Bis-acrylamide solution (29:1)

1.5M Tris pH 8.8 buffer

ddH2O

10% SDS

10% APS

TEMED

0.5 M Tris pH 6.8 buffer

Process:

Table 1

| | |
|---|---|
| 40 % Acrylamide /Bis-acrylamide solution (29:1) | 3ml |
| 1.5M Tris pH 8.8 buffer (pH8.8) | 2.5ml |
| ddH2O | 4.3ml |
| 10% SDS | 100µl |
| 10% APS | 100µl |
| TEMED | 10µl |

Table 2

| | |
|---|---|
| 40 % Acrylamide /Bis-acrylamide solution (29:1) | 0.5ml |
| 0.5 M Tris pH 6.8 buffer | 1.25ml |
| ddH2O | 3.2ml |
| 10% SDS | 50µl |
| 10% APS | 50µl |
| TEMED | 5µl |

1. The gels are polymerized in an appropriate container, such a beaker, by mixing the different solutions detailed in the table 1 and casting them into the gel casting system . First a separating gel is casted and subsequently after the separating gel has polymerised the stacking gel is casted on top. This will generate a discontinuous gel. The upper or stacking gel will allow the concentration of the sample before entering the separating gel.

2. The casting of the gels require to be swiftly performed, as polymerization starts immediately after the TEMED it is added to the mixture, therefore it should be the last solution to be added.

3. A demonstrator will show you how the electrophoretic system is assembled. The system basically consists in a tank with an inner scaffold that will hold vertically the SDS PAGE gels. The tank will be connected to an electric power supply.

4. Before assembling the system, the gels need to be casted by mixing all ingredients shown in the table 2 into a mould built between two glass plates. The demonstrator will show how this is done. Once the gel has polymerized, it can be transferred to the electrophoretic system.

5. load protein onto the gel. Run the gel.